

Title: Managing Grubs in Turf with Persistent Nematodes

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Abstract:

White grubs are the most prevalent insect pests of both high and low maintenance turfgrass in New York State. Grub control in turf with insecticides is complicated because the lower toxicity insecticides need to be applied before the need for their application can be assessed and the two labeled curative insecticides are highly toxic and have variable efficacy against white grubs. Demand is increasing among homeowners, grounds managers and golf course superintendents for low-impact grub control practices and products, such as biological control. Entomopathogenic nematodes (EPNs) are the only effective biological control for grubs. With support of the NYSIPM Program, and the Battle Island State Park Golf Course, the feasibility of utilizing native NY EPNs which retain their ability to persist in the turf system to augment existing nematode populations to suppress grub populations over multiple years was tested in the summer/fall 2013. Fifteen fairways were divided down the middle and EPN combinations (*S. carpocapsae* x *H. bacteriophora*, *S. feltiae* x *H. bacteriophora*, and *S. carpocapsae* x *S. feltiae*) were applied to one-half of the length of each fairway (5 fairways/combination).

Early results report the successful inoculation of these native EPNs into the turf environment. However, there was no difference in the white grub populations between the untreated and treated portions of the fairways. These observations suggest that white grubs have two susceptible windows to attack by native EPNs. Those windows are early larval instars before the larvae has formed a feeding cell and when the late instars leave their earthen cells in the fall to move deeper in the soil to overwinter. A second sampling for grubs and treatment impact will occur in the spring of 2014 before insect pupation.

Background and Justification:

White grubs are the most prevalent insect pests of both high and low maintenance turfgrass in New York State. Their damage ranges from loss of a small percentage of roots that may weaken a turf stand and allow encroachment of weeds and bare spots, to total loss of turf cover resulting from massive root destruction. Furthermore, substantial damage from predators such as raccoons and skunks destroying turf in search for grubs occurs when either high or low populations of grubs are present.

Good IPM for grubs in turf with insecticides is complicated by two factors:

- 1) To be efficacious, the products of lower toxicity (e.g. chlorantraniliprole, imidacloprid) need to be applied preventatively, before the need for their application can be assessed through sampling and comparison with treatment thresholds.

- 2) The two labeled curative products are both of high toxicity. Trichlorfon is experiencing an increased failure rate and carbaryl has always demonstrated low and/or variable efficacy against white grubs.

The demand is high for low-impact grub control practices and products, such as biological control. Schools in NY are no longer allowed to use synthetic pesticides without an emergency exemption, while being responsible for valuable athletic field turf and the safety for the individuals playing on it. Homeowners, grounds managers and golf course superintendents are also interested in low impact alternatives.

Entomopathogenic nematodes (EPNs) are the only effective biological control for grubs. Commercially purchased nematodes are not commonly used because of their high cost and variable efficacy. Applications are timed after a problem is detected and commercial nematodes only remain active for a relatively short time (2-4weeks) due to intensive selection for a fast acting nematode. Application of commercial nematodes can be best viewed as a short persistence biopesticide. The concept of utilizing native NY strains with the ability to persist in the NY soil environment has been developed in Northern NY as a successful biological control strategy for alfalfa snout beetle, *Otiorhynchus ligustici*. These native NY strains of EPNs are adapted to NY conditions since they occur naturally in NY agricultural systems. Research over the past 25+ years with these native nematodes has shown that a single application will inoculate the soil and these nematodes will persist for many years. The size of the EPN soil population is a direct reflection of the residual soil insect population level or the rate of insect invasion. The turf system is typically home to an array of susceptible insect hosts in addition to the white grub complex and we anticipate long-term persistence in the turf environment. This concept can be best viewed as a classical approach to biological control and a direct opposite approach from the application of commercial nematodes as a biopesticide.

The Shields Lab has native NY strains of *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* in culture and these species are the same species which are commonly purchased from commercial suppliers. Great care is taken to preserve the ability of these NY strains to persist under NY conditions. In addition, the Shields Lab has developed a nematode mass rearing method that dramatically reduces the cost of the nematodes and application techniques which are compatible with commercial pesticide sprayers. The efficacy of these persistent native strains of EPNs needs to be tested against the array of soil insects commonly causing damage within the NY turf environment, with white grubs being the target of this project.

Objectives:

- 1) Evaluate the efficacy of 3 different nematode species combinations against white grubs in the commercial turf environment.
- 2) Document the persistence of these native NY nematode strains in the commercial turf environment.
- 3) Evaluate the project's objectives and publicize the results.

Procedures:

Research for this project was conducted at the Battle Island State Park Golf Course (Figure1), an 18-hole public course in Fulton, NY. The facility has a long history of grub infestation, mainly Japanese beetles, and the superintendent was an excellent cooperater with a keen interest in the project.



Figure 1. Battle Island State Park Golf Course – Fulton, NY

Objective 1:

A survey of 15 fairways based on acreage and grub history was conducted July 24-29 to determine presence of naturally occurring nematodes. Two thousand forty soil samples (Figure 2) were collected across the 15 fairways and returned to Cornell University where each sample was analyzed utilizing a *Galleria*-based laboratory assay. The location of each soil sample collected for the bioassay was recorded with GPS. Nematode species detected in each soil sample was recorded and frequency of nematode occurrence was recorded from each fairway.



Figure 2. Survey for presence of naturally occurring nematode

Assay results showed an existing EPN population (*S. carpocapsae*, *H. bacteriophora*, and *S. feltiae*) throughout the golf course at variable levels. The assignment of the different EPN treatments to individual fairways was adjusted to accommodate the natural occurring nematode population. The treatments consisted of three different combinations of nematode species (*S. carpocapsae* x *H. bacteriophora*, *S. feltiae* x *H. bacteriophora*, and *S. carpocapsae* x *S. feltiae*) (Table 1);

Table1.Nematode Species Combination Assignment

Sc/Hb	Sf/Hb	Sc/Sf
Hole 8	Hole 2	Hole 1
Hole 10	Hole 7	Hole 3
Hole 13	Hole 11	Hole 4
Hole 14	Hole 15	Hole 5
Hole 16	Hole 18	Hole 12

Each fairway was divided lengthways down the middle creating a treated and untreated plot the full length of each fairway. The EPN treatment was applied to one of the fairway halves while the remaining half remained untreated and utilized as the untreated check. EPN infective juveniles (IJ) were applied at a rate of 200 million per species per acre of fairway. Since each fairway received a two-species combination, the total number of IJs per acre of fairway was 400 million.

Treatment combination, *S. feltiae* plus *H. bacteriophora* were applied to the fairways on August 16, 2013, treatment combination *S. carpocapsae* plus *H. bacteriophora* were applied to the fairways on August 19, 2013, and treatment combination *S. carpocapsae* plus *S. feltiae* were applied to the fairways on August 20, 2013. For each application event, infective juveniles were washed from rearing containers (Figure 3), and strained twice through screens before being added to the 50 gallon spray tank inside the truck (Figure 4 & 5).



Figure 3. Wax worm cups infected with IJs prior to wash



Figure 4. 50 gallon water tanks used in EPN applications



Figure 5. Filling water tanks with EPNs using a pump

One spray tank was filled with nematodes and the second tank was filled with water. Nematodes were applied using a spray boom fitted with 0010 fertilizer stream nozzles (screens removed). A second set of flat fan nozzles were used to apply water behind the nematode application to wash any nematodes off of the turf and onto the soil surface (Figure 6).



Figure 6. Truck with spray boom attached applying EPNs to fairways

In early October 2013, each fairway was sampled for grubs. An equal number of samples were collected and searched for grubs from both the treated and untreated sides of the fairway. Grubs were collected, location identified and returned to Cornell for identification. Using the earlier GPS locations from the soil bioassay, a designated number of locations were determined for each fairway and golf course cup cutters were used to retrieve the soil samples (Figure 7 & 8).



Figure 7. Golf course cup cutter used to retrieve soil for grub assessment



Figure 8. Checking soil for grubs

Objective 2:

To document EPN persistence under turf conditions, nematode soil samples were taken on the treated side of the fairway at each of the grub sampling locations. At each grub sampling location, four nematode soil cores were collected at four equal points around the grub sample. Soil cores were returned to Cornell and analyzed utilizing a *Galleria*-based laboratory assay (Figure 9).



Figure 9. Soil sampling for EPN establishment surrounding hole assayed for grub populations

A second nematode survey will be conducted in the spring of 2014 at the same time as the spring grub sampling to document the nematode persistence after a typical NY winter.

Objective 3:

The impact of using these native persistent nematodes on the white grub complex will be evaluated using statistics. The persistence of the native nematodes strains will be measured by comparing the nematode population levels 42 days after application to the population levels measured 270 days after application. Subsequent persistence and efficacy will also be evaluated in future years. Results will be publicized, at a minimum, via ShortCutt (Cornell's weekly phone call and newsletter), CUTT (Cornell University Turfgrass Times), and the NYSIPM website.

Results:

Battle Island State Park Golf Course Fairway Survey

Assay results showed an existing EPN population (*Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*) at variable levels within the turfgrass area of the golf course (Table 2). The number of samples removed was determined by the size of each fairway and the number of samples was adjusted according (Figure 10). Samples were collected over a four day period and each sample point was recorded using a GPS unit. Table 1 represents the results from the initial pre-treatment survey of the golf course.

Table2. EPN populations found in assays represent one of the three species used in the project.

# Fairway	# Cores	% EPN Positive	# Fairway	# Cores	% EPN Positive	# Fairway	# Cores	% EPN Positive
1	120	13	7	140	41	13	80	33
2	100	05	8	120	22	14	180	33
3	160	11	10	140	10	15	140	33
4	90	19	11	120	22	16	150	49

5	120	07		12	200	13		18	180	48
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Figure 10. Battle Island State Golf Course Fairways

Fall 2013 Grub Population Assay

The October assays for grub densities within the treated and untreated halves of the represented fairways were inconclusive. Results showed little to no variation between the treated and untreated halves for each fairway and species combination (Table 3).

Table 3. Battle Island Fall 2013 Grub Assay

Sc/Sf Combination					
# Fairway	# Points	# JB Grubs (UNT)	# Other Grubs (UNT)	# JB Grubs (TRT)	# Other Grubs (TRT)
1	20	3	0	1	0
3	30	15	0	13	0
4	15	6	0	9	0
5	15	8	0	4	0
12	30	13	2 EC	17	0
Total	110	45	2 EC	44	0
Sc/Hb Combination					
# Fairway	# Points	# JB Grubs (UNT)	# Other Grubs (UNT)	# JB Grubs (TRT)	# Other Grubs (TRT)
8	25	9	0	15	0

10	20	5	1 EC	6	0
13	15	5	0	10	2 Ec
14	25	1	1 EC	7	2 EC
16	25	7	1 EC	14	0
Total	110	27	3 EC	52	4 EC
Sf/Hb Combination					
# Fairway	# Points	# JB Grubs (UNT)	# Other Grubs (UNT)	# JB Grubs (TRT)	# Other Grubs (TRT)
2	25	3	0	8	1 EC
7	20	4	0	5	1 AGB
11	15	5	0	5	0
15	15	5	0	7	0
18	30	12	1 AGB 1 EC	21	0
Total	105	29	1 AGB 1 EC	46	1 AGB 1 EC

(JB=Japanese beetle, EC=European chafer, AGB=Asiatic garden beetle)

Fall 2013 EPN Establishment Assay

Soil cores from each treated fairway were bioassayed for the presence of EPNs 42-48 days after application. At each sample point (GPS point matching grub assessment location) four samples were removed (4ft²area) and samples results were recorded (Table 4).

Table 4. Battle Island EPN Establishment Assay Fall 2013

Sf/Hb Combination					
# Fairway	# Points	# Cores	% Positive Cores-Species		% Positive Cores-Hole
			Sf	Hb	
2	25	100	33	6	36
7	20	80	24	5	31
11	15	60	13	3	17
15	15	60	32	0	27
18	30	120	13	14	26
Sc/Hb Combination					
# Fairway	# Points	# Cores	% Positive Cores-Species		% Positive Cores-Hole
			Sc	Hb	
8	25	100	8	6	13
10	20	80	16	5	21
13	15	60	17	23	28
14	25	100	5	26	24

116	25	100	14	19	28
Sc/Sf Combination					
# Fairway	# Points	# Cores	% Positive Cores-Species		% Positive Cores-Hole
			Sc	Sf	
1	20	80	5	23	27
3	30	120	9	28	25
4	15	60	2	38	37
5	15	60	10	12	18
12	30	120	9	13	21

All the applied nematodes became established in each fairway at levels that are consistent with our previous research. EPN levels below 20% are a little low based on previous research, but this is usually a result of sampling error. Frequently, spring sampling will yield EPN numbers higher than the sampling 30-42 days after application. Nematodes are applied in streams that are separated by 24' and a couple of months are required for the nematodes to fill in between the application streams.

Discussion:

The lack of difference in white grub numbers 42 days after nematode application is disappointing but not surprising. During grub sampling, earthen cell around the large grubs were observed and it appeared to protect the grubs from nematode attack. Commercial nematodes that are applied in the September-October period to attack the large damaging grubs require irrigation after application to assist the movement of nematodes into these cells to be effective. It would appear that there are two different windows for native EPNs persisting in the soil to attack white grubs. The first window is the very small grubs before an earthen cell is formed. The high EPN numbers on some fairways collected during the pre-sample strongly suggest nematode recycling in these small larvae. The second window would be when the large grubs move down in the soil profile to overwinter. The native NY nematodes applied remain infective and mobile down to 10 degrees C for both of the *Steinernema sp.* and down to 15 degrees C for the *Heterorhabditis sp.* The reason for the planned late spring grub sampling is to see if this second window is also an effective attack window for native nematodes persisting in the soil profile

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Samples of Resources Developed:

See figures and digital photographs.

Appendix:**Battle Island EPN Establishment Assay Data Summary Fall 2013**

Sc/Sf Combination										
# Fairway	# Points	# Cores	# Points No EPN Presence	% Negative Points	# Positive Sc Cores		# Positive Sf Cores		% Positive Cores	
					2"	4"	2"	4"	Sc	Sf
1	20	80	6	30	4	0	6	12	3	23
3	30	120	3	10	7	4	12	22	9	28
4	15	60	2	13	1	0	11	12	2	38
5	15	60	6	40	6	0	2	5	10	12
12	30	120	13	43	11	0	8	8	9	13
Total	110	440	40	27	29	4	39	59	8	22
Sc/Hb Combination										
# Fairway	# Points	# Cores	# Points No EPN Presence	% Negative Points	# Positive Sc Cores		# Positive Hb Cores		% Positive Cores	
					2"	4"	2"	4"	Sc	Hb
8	25	100	14	56	8	0	3	3	8	6
10	20	80	9	45	5	8	3	1	16	5
13	15	60	3	20	10	0	5	9	17	23
14	25	100	8	32	5	0	13	13	5	26
16	25	100	6	24	12	2	6	13	14	19
Total	110	440	40	36	40	10	30	39	11	16
Sf/Hb Combination										
# Fairway	# Points	# Cores	# Points No EPN Presence	% Negative Points	# Positive Sf Cores		# Positive Hb Cores		% Positive Cores	
					2"	4"	2"	4"	Sf	Hb
2	25	100	4	16	19	14	1	5	33	6
7	20	80	5	25	9	10	3	1	24	5
11	15	60	6	40	5	3	1	1	13	3
15	15	60	5	33	0	0	9	5	0	23
18	30	120	6	20	14	2	5	12	13	14
Total	105	420	39	37	47	29	19	24	18	10